Hindawi
Journal of Zoological Systematics and Evolutionary Research
Volume 2022, Article ID 3618288, 5 pages
https://doi.org/10.1155/2022/3618288

Research Article

Embryonic Development and Histological Analysis of Skeletal Muscles of *Tenuidactylus caspius* (*Eichwald*, 1831) Lizards (Reptilia: Squamata)

Janbakhish Najafov,[1] Ramin Hashimov ,[2] Rovshan Khalilov ,[3] and Parviz Vahedi [4]

[1]*Department of Zoology and Physiology, Baku State University, 23 Academician Z.Khalilov Str., Baku AZ 1148, Azerbaijan*
[2]*Department of Medical Biology and Genetics, Azerbaijan Medical University, 167 S. Vurgun Str., Baku AZ 1022, Azerbaijan*
[3]*Department of Biophysics and Biochemistry, Baku State University, Baku AZ 1148, Azerbaijan*
[4]*Department of Anatomical Sciences, Maragheh University of Medical Sciences, Maragheh 78151-55158, Iran*

Correspondence should be addressed to Ramin Hashimov; raminhesimov@mail.com and Parviz Vahedi; pa.vahedi@yahoo.com

Received 6 July 2022; Revised 4 October 2022; Accepted 18 October 2022; Published 28 October 2022

Academic Editor: Muhammad Muddassir Ali

During embryonic development of the Caspian thin-toed gecko migration, formation of myoblast and myosatellite cells occurs in the cranial-distal direction. Somite formation begins in the body part close to the skull and ends in the tail. The time of separation of somites from the proximal mesoderm depends on the temperature of the air and the substrate. Myoblast cells reach their targets and are connected, and the membranes in the area of their contact are destroyed. Myoblast's fusion creates myosymplasts. The intermediate stage is observed after the formation of small myosymplasts. After that, the chain shape of myosymplasts are transformed into an intermediate plaque form. At this intermediate stage, the number of a nucleus is small, the shape of the nucleus differs from each other, and the location of the nucleus varies. Afterward, the connection of the intermediate forms with each other and with myoblasts forms a rounded shape, where the initial development of myotubules takes place. A fully formed myotubular and myosatellite cells are surrounded by a basal membrane and shape a muscle fiber. The skeletal muscles of the adult Caspian thin-toed gecko are mainly composed of white fibers. Thus, it allows the gecko to move very fast in a short time. Due to the small number of mitochondria in the myotubes, oxygen gas demand is decreased and the body is prevented from overheating.

1. Introduction

The Caspian thin-toed gecko is a widespread lizard in Azerbaijan. This lizard can be seen at altitudes up to 2400 m. It is enough to have shelter in these areas so that the lizard can adapt to that area. It is enough for the lizard to have shelter to adapt to the terrain. This lizard does not stray far from the shelter. Caspian thin-toed gecko has high adaptability. Geckos have a large synanthropic population in urban areas. They are mostly active at night. Appropriate methodological approaches were used to study the histological and cytological structure of the skeletal muscles of this lizard. When we catch the lizard for histological studies, the population density was taken into account. Microfilaments are found in all tissues of the Caspian thin-toed gecko. A high rate of microfilaments in muscle tissue provides muscle contraction. A muscular contraction of a certain part of the lizard body is manifested with the help of intracellular objects. During embryonic development of the geckos, migration and formation of the myoblast and myosatellite cells transpire through the interaction of genetic, endocrine, and environmental factors. The development and differentiation occur in the cranial-distal direction. From the myotomes of the paraxial mesoderm, the migration of myoblast cells takes place under the control of the connective tissue. Achieved targets of myoblasts cells join together with the formation of myosimplasts in the chain shape. Thereafter, the chain shape of myosimplasts transforms into plaque form. Afterward, the

Figure 1: Developed gecko's egg before oviposition.

connection of the intermediate forms with each other and with myoblasts leads nuclei to form a rounded shape where initial myotubular development takes place. During subsequent development, the growing microtubules in the myotubes displace the nuclei, and the nuclei change their shape and become partially elongated. A fully formed myotubular and myosatellite cells are surrounded by a basal membrane and shape a muscle fiber. The skeletal muscles of the adult Caspian thin-toed gecko are mainly composed of white fibers. Thus, it allows the gecko to move very fast in a short time. Due to the small number of mitochondria in the myotubes, oxygen gas demand is decreased and the body is prevented from overheating. The endomysium and perimysium are well-formed in the skeletal muscles of the gecko; however, connective tissue fibers are weakly developed in the epithelium.

2. Materials and Methods

The research was conducted at the Department of Medical Biology and Genetics and the Department of Cytology, Histology, and Embryology of AMU from 2009 to 2022. 11 adults and 22 eggs of the Caspian thin-toed gecko *Tenuidactylus caspius* (Eichwald, 1831) [1] were used for histological research which is widespread in the territory of the Republic of Azerbaijan. The object was preliminarily fixed in a 10% formalin solution. Paraffin-embedded tissue blocks were prepared from these samples and made 3-5 μm thick incisions. Paraffin is a water-insoluble substance. Since the tissue contains water, it is impregnated with paraffin. For water extraction from the tissue, we used ethyl alcohol. Firstly, 70%, then 95%, and finally, 100% ethanol are used during this process. Careless water extraction from the tissue may cause shrinking and hardening. After dehydration, the tissue should be transparent. The substances used for this are xylene, toluene, benzene, chloroform, and limonene. After tissue clearing, it is embedded in paraffin, for microtome machine usage. Blocks are made by placing a tissue on melting paraffin at a temperature of 55-60°C. After blocking, the tissue is stored in a cool place for a while. The block is put on the stage of the microtome machine, and incisions are made with a thickness of 3-5 μm. The slices are separated from the block and placed in distilled water prepared inside a container with a dark bottom at a temperature of 35°C. For solution preparation, add 5 ml of 95% alcohol for every 100 ml of its volume. As soon as the cuts in the water take a straight shape, they are captured by the glass. Glass and mixture are dried at a temperature of 40-45°C. In this case, part of the paraffin melts and separates from the tissue and provides adhesion of tissue to the glass. The preparation of the dried glass is placed in two xylene baths for 5 minutes each. It is essential to remove paraffin and make the tissue transparent. Subsequently, the tissue is placed in containers with 100%, 95%, and 70% alcohol and aged for 5 minutes each. The sample is placed in distilled water for 10 minutes. The tissue is now ready to be dyed. Hematoxylin-eosin dyes are used to stain muscle tissue. The tissue is kept in the hematoxylin-eosin dyed for 4 minutes and then placed under running water for 1-2 minutes. Then, the muscle tissue covered with a coverslip is examined under a light microscope. During staining, the muscle fibers turn reddish-pink and the nucleus turns blue.

3. Results and Discussion

The development of the muscle tissue of the Caspian thin-toed gecko begins with the differentiation of somites and the formation of myotomes. Somitogenesis develops through the interaction of genetic [2], endocrine, and environmental factors [3]. Micromorphological observations show that the development and differentiation of somites in the embryo proceed in the craniodistal direction. Although the formation of the first somites occurs in a short time, subsequent somite formation is somewhat slower. Somite formation begins in the oviduct of female geckos. Before oviposition, the egg had a soft and sticky shell (Figure 1). At that time, amnion completely covers the embryo and 12-14 somites were observed in the embryo.

Depending on the air temperature (24-35°C), the substrate (28-36°C), and 50–70% humidity, the time of somite separation from the proximal mesoderm may vary. The complete development of each somite is encouraged by the covering of its surface with a layer of epithelium [4]. Somite formation begins in the body part close to the skull and ends in the tail. After segmentation, the somitomeres are covered by epithelial cells [5]. The first somites start visibly in the first week of embryonic development. In this case, the embryonic disc is located eccentrically just outside the center of the animal's pole. Somites in the tail of the embryo are smaller than those placed in the body and head. At this stage, the somites near the skull of the embryo become sizable, while the somites which formed in the tail develop weakly. The rapid growth of somites close to the brain is associated with the process of preparation for the onset of myotomies, sclerotomes, and dermatomes and their rapid innervation by the central nervous system. Firstly, the somites which are close to the head begin to differentiate and become relatively thinner and shorter. While the somites on the front of the embryo transform into myotomes, the somite formation process continues in the tail. The initiation of somites into myotomes, sclerotomes, and

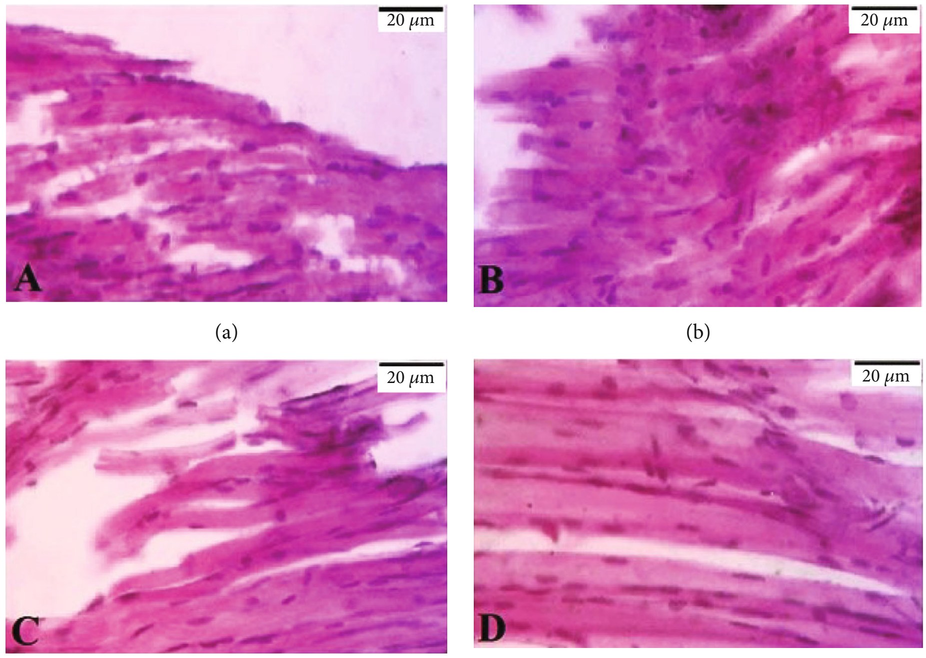

FIGURE 2: The myogenesis of lizard tissues (stained with hematoxylin-eosin). (a) Formation of myosymplasts (7th day of postoviposition). (b) Organization of intermediate plate stage (9th day of postoviposition). (c) Primary myotubules (18th day of postoviposition, taken from the forelimb). (d) Myotubules (30th day of postoviposition, taken from the forelimb).

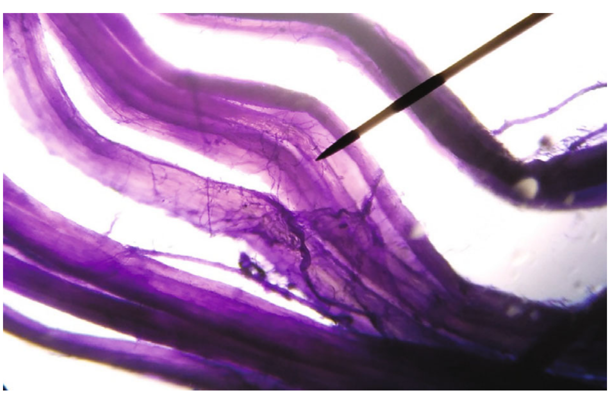

FIGURE 3: In the skeletal muscles of the gecko, connective tissue fibers of epimysium are weakly developed.

dermatomes in lizards also starts from the front of the body and ends in the tail.

Myoblasts are formed from the myotomes of the paraxial mesoderm in gecko somites. These cells are proliferatively active [6] and can divide and reproduce by mitosis [7]. Myoblasts migrate under the control of the connective tissue [8]. When myoblast cells reach a target, the membranes in the area of their contact are destroyed and transformed into the sarcoplasmic reticulum [9]. Myoblast's fusion creates chain-like myosymplasts (Figure 2(a)). The intermediate stage is observed after the formation of small myosymplasts. At this intermediate stage, the number of a nucleus is small, and their shape differs from each other (Figure 2(b)). The location of the nucleus in the plate-like intermediate stage, which is wider than the myotubule, is also different. After, plate-like transitional stages combine with myoblasts to form the primary myotubule (Figure 2(c)). The initial muscular tube contains many nuclei, which are initially based in the center of the tube. The first myofibrils inside the primary muscular tube are located on the periphery of the tube. To perform functions well, myofibrils, which then grow in myotubules, push the nucleus into the edges. The nucleus located close to the sarcolemma transform into a partially elongated shape (Figure 2(d)). If the nucleus remained in the center [10], it would prevent the accumulation of skeletal muscle fibers and slow down the differentiation of muscle fibers [11].

Some myoblasts are not involved in the formation of myotubules [12]. These cells remain between the basement membrane [13] and the plasma membrane of the muscle fiber [14], creating myosatellite cells [15]. Satellite cells are more common in the muscle fibers of the lizard tail. When a lizard's tail is ripped off for some reason [16], myosatellite are actively involved in muscle regeneration [17]. On the other hand, along with myosatellite, the red bone marrow [18] cells in the vertebrae also divide and participate in the repair of damaged transverse skeletal muscle tissue. The nucleus divides and reproduces by mitosis inside the myotubule. The fully formed myotubule and satellite cells are surrounded by a basement membrane and create the basis of the muscle fibers. The sarcolemma of the muscle fibers of the lizard generally maintains the same structure as the cell membrane [19]. At every beginning and end of the muscle fiber, the sarcolemma fuses with tendon fibrils. Being localized close to the myotubular surface and under the basement membrane, they are not easily visible under a light microscope. These cells are seen under an electron microscope and are spindle-shaped [20].

The organs that produce the most heat in the lizard are the muscles and the liver. The skeletal muscles of the Caspian thin-toed gecko are mainly composed of white fibers. This allows the gecko to move very fast in a short time. Having white-colored fibers in the skeletal muscle is an evolutionary advantage and provides thermal protection during movements at high temperatures. Muscles with white fibers are ideal for quick contractions of short duration. The reason why they quickly get tired is the small number of mitochondria. Due to the small number of mitochondria in the Caspian thin-toed gecko and most of the skeletal muscle mass being light-colored which makes this species better adapted to extreme conditions, hypoxia prevents the body from overheating. White fibers contain a large amount of glycogen and myofibrils and are larger than red fibers. The diameter of the muscle fiber varies depending on, age, size, and movement activity. The diameter of the muscle fiber varies in different parts of each lizard. Sarcomeres of skeletal muscle fibers are seen in lizards quite well. Each skeletal muscle fiber is surrounded by an endomysium and separated from the other. In other words, the transversalis muscle fiber never comes into contact with other muscles next to it.

When we examined the cross-section of muscles, we saw that endomysium is fused. It is also connected to the perimysium by intermittent perimysial junctional plates. Perimysium is a sheath of connective tissue, and it is significantly thicker than the endomysium. For muscle organ formation [21], bundles of muscle fibers fuse with the epithelium. The epimysium consists of fibrous connective tissue that surrounds muscle that links to the perimysium. Examining the skeletal muscles of the limbs, we observed that the endomysium and perimysium are well-formed in the skeletal muscles of the gecko; however, connective tissue fibers of epimysium are weakly developed (Figure 3).

Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors are thankful to Baku State University for providing all necessary research facilities to carry out this research.

References

[1] S. H. Yousefkhani, "Habitat suitability prediction of Tenuidactylus caspius (Eichwald, 1831) (Reptilia: Gekkonidae) in Iran," *Journal of Biological Studies*, vol. 2, no. 1, pp. 4–8, 2019.

[2] A. Roll-Mecak, "The tubulin code in microtubule dynamics and information encoding," *Developmental Cell*, vol. 54, no. 1, pp. 7–20, 2020.

[3] Y. S. Bogliotti, W. J. Vilarino, M. Okamura et al., "Efficient derivation of stable primed pluripotent embryonic stem cells from bovine blastocysts," *Proceedings of the National Academy of Sciences*, vol. 115, no. 9, pp. 2090–2095, 2018.

[4] P. Vahedi, R. Moghaddamshahabi, T. J. Webster et al., "The use of infrapatellar fat pad-derived Mesenchymal stem cells in Articular cartilage regeneration: A review," *International Journal of Molecular Sciences*, vol. 22, no. 17, p. 9215, 2021.

[5] A. Najafov Dj and R. T. Hashimov, "Morphogenesis of somatic muscles in reptiles in early embryogenesis," *Morphology*, vol. 145, no. 3, p. 136, 2014.

[6] L. Alibardi, "Review: biological and molecular differences between tail regeneration and limb scarring in lizard: an inspiring model addressing limb regeneration in amniotes," *J. Exp. Zool. (Mol. Dev. Evol.)*, vol. 328, no. 6, pp. 493–514, 2017.

[7] E. A. Adib, L. J. Smithson, and C. A. Collins, "An axonal stress response pathway: degenerative and regenerative signaling by DLK," *Current Opinion in Neurobiology*, vol. 53, pp. 110–119, 2018.

[8] H. Yin, F. Price, and M. A. Rudnicki, "Satellite cells and the muscle stem cell niche," *Physiological Reviews*, vol. 93, no. 1, pp. 23–67, 2013.

[9] S. K. Verma, E. Leikina, K. Melikov et al., "Machinery that fuses osteoclast membranes," *The Journal of Biological Chemistry*, vol. 293, no. 1, pp. 254–270, 2018.

[10] J. Liu, Z. P. Huang, M. Nie et al., "Regulation of myonuclear positioning and muscle function by the skeletal muscle-specific CIP protein," *Proceedings of the National Academy of Sciences*, vol. 117, no. 32, pp. 19254–19265, 2020.

[11] E. T. Spiliotis and K. Nakos, "Cellular functions of actin- and microtubule-associated septins," *Current Biology*, vol. 31, no. 10, pp. R651–R666, 2021.

[12] A. Ramkumar, B. Y. Jong, and K. M. Ori-McKenney, "ReMAPping the microtubule landscape: how phosphorylation dictates the activities of microtubule-associated proteins," *Developmental Dynamics*, vol. 247, no. 1, pp. 138–155, 2018.

[13] N. K. Singh, H. J. Lee, D. K. Jeong, H. S. Arun, L. Sharma, and I. H. Hwang, "Myogenic satellite cells and its application in animals - a review," *Asian-Australasian Journal of Animal Sciences*, vol. 22, no. 10, pp. 1447–1460, 2009.

[14] C. M. Fan, L. Li, M. E. Rozo, and C. Lepper, "Making skeletal muscle from progenitor and stem cells: development versus regeneration," *Wiley Interdisciplinary Reviews: Developmental Biology*, vol. 1, no. 3, pp. 315–327, 2012.

[15] T. P. Lozito and R. S. Tuan, "Lizard tail skeletal regeneration combines aspects of fracture healing and blastema-based regeneration," *Development*, vol. 143, no. 16, pp. 2946–2957, 2016.

[16] A. M. Bauer, R. Masroor, J. Titus-Mcquillan, M. P. Heinicke, J. D. Daza, and T. R. Jackman, "A preliminary phylogeny of the Palearctic naked-toed geckos (Reptilia: Squamata: Gekkonidae) with taxonomic implications," *Zootaxa*, vol. 3599, no. 4, pp. 301–324, 2013.

[17] V. Meyer, M. R. Preest, and S. M. Lochetto, "Physiology of original and regenerated lizard tails," *Herpetologica*, vol. 58, no. 1, pp. 75–86, 2002.

[18] J. J. Ramírez-Espinosa, L. González-Dávalos, A. Shimada, E. Pinã, A. Varela-Echavarria, and O. Mora, "Bovine (Bos

taurus) Bone marrow mesenchymal cell differentiation to adipogenic and myogenic lineages," *Cells, Tissues, Organs*, vol. 201, no. 1, pp. 51–64, 2016.

[19] S. Kreissl, A. Uber, and S. Harzsch, "Muscle precursor cells in the developing limbs of two isopods (Crustacea, Peracarida): an immunohistochemical study using a novel monoclonal antibody against myosin heavy chain," *Development Genes and Evolution*, vol. 218, no. 5, pp. 253–265, 2008.

[20] D. A. Gordeeva, N. B. Ananjeva, and D. V. Korost, "Autotomy and regeneration in squamate reptiles (Squamata, Reptilia): defensive behavior strategies and morphological characteristics (using computer microtomography methods)," *Izvestiya Akademii Nauk, Seriya Biologicheskaya*, vol. 47, no. 4, pp. 389–398, 2020.

[21] J. Wang, Y. Fan, S. Dube et al., "Myofibril assembly and the roles of the ubiquitin proteasome system," *Cytoskeleton*, vol. 77, no. 10, pp. 456–479, 2020.